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CIRCULATING BLOOD VOLUMES IN THE LABORATORY*

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The determination of circulating blood volumes has become an important addition to the series of techniques performed in the clinical laboratory. You will likely have many requests for such determination in the future. Several excellent articles have appeared in medical and laboratory journals describing the conduct and technique, and the surgeons and physicians are quickly realizing the value and utility of these tests.

The primary purpose of this paper is to correlate much of what has been printed into a concise and complete technique, with a method for photolometric calculation to further guarantee such results. Briefly, the reading of plasma samples in the photoelectric colorimeter may encounter such hazards as turbidity, of which lipemia is only one example. The accuracy of reading plasma, utilizing the method advanced by O. H Gaebler for such contingencies, cannot be emphasized too much.

PRINCIPLE. The basic principle of this method is simple. A known amount of dye is injected intravenously into one arm of the patient. After a definite interval of time, blood is withdrawn from the opposite arm, and the dilution of the dye in the plasma is calculated on the photoelectric colorimeter. The dilution multiplied by the amount of dye given is the total plasma volume.

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PREPARATION. 1. Three 21 ga. syringe needles and 10 cc. syringes should be kept especially for this test. Sharp needles will facilitate the injection of the dye, helping to avoid puncturing the opposite walls of the veins.

One syringe is calibrated by adding 5 cc. of distilled water with a volumetric pipette, being sure not to have a needle attached to the syringe. A mark may be filed on the plunger close to the barrel. Any error inherent in this method will not be marked.

Two uncalibrated syringes are used for collection of hematocrits.

THE NEEDLE ADAPTER BEVELS OF THE SYRINGES MUST BE EXAMINED EACH TIME THESE ARE USED FOR TESTS; THIS WILL INSURE BLOOD COLLECTED PRECISELY ON TIME.

Rinse each syringe with sterile normal saline prior to immediate use.

2. Pipette 1 cc. of 1.1 percent sodium oxalate solution into four Sanford-Magath hematocrit tubes. Label two tubes with the numeral 1, and the others with numeral 2.

3. This test will require two persons for successful handling of the test.

4. Patients in acute shock may have a poor venous circulation. The veins usually collapse, making it difficult to secure accurate plasma samples. The arteries may be used, but this is not recommended by most surgeons (to our knowledge). If the patient is in an acute state the results will not be reliable, however, unless the artery is used by the person performing the test.

TECHNIQUE. 1. Fill a carefully calibrated syringe with exactly 5 cc. of Evans Blue dye (T-1824). Also, have the four hematocrit tubes ready for use.

2. Take over 10 cc. of blood from one arm of the patient, being careful to use the tourniquet for only a second or two. (The tourniquet is almost immediately released to prevent stasis with concomitant inaccurate hematocrit values.) This blood is put into the number 1 hematocrit tubes, which are inverted four or five times.

3. The assistant will slip the syringe containing dye into the needle as the blood syringe is withdrawn from it.

NOTE THE EXACT TIME AT THE BEGINNING OF THE DYE INJECTION.

Inject the dye slowly and rinse the syringe four or five times with the blood of the patient. This will wash nearly all traces of the dye into the venous system.

4. In EXACTLY TEN MINUTES after the injection (or FIFTEEN MINUTES for patients with congenital heart disease or cardiac decompensation), withdraw over 10 cc. of blood

TABLE OF Lr VALUES

(Computed as Logarith 100 Minus Logarith Photoelectric Reading)

Photocolorimeter Reading in %	Lr Value:
2	1.6990
3	1.5229
4	1.3979
5	1.3010
6	1.2218
7	1.1549
8	1.0969
9	1.0458
10	1.0000
11	0.9586
12	0.9208
13	0.8861
14	0.8539
15	0.8239
16	0.7959
17	0.7696
18	0.7447
19	0.7212
20	0.6990
21	0.6778
22	0.6576
23	0.6383
24	0.6198
25	0.6021
26	0.5850
27	0.5686
28	0.5528
29	0.5376
30	0.5229
31	0.5086
32	0.4949
33	0.4815
34	0.4685
35	0.4559
36	0.4437
37	0.4318
38	0.4202
39	0.4089
40	0.3979
41	0.3872
42	0.3768
43	0.3665
44	0.3565
45	0.3468
46	0.3382
47	0.3279
48	0.3188
49	0.3098
50	0.3010

Photocolorimeter Reading in %	Lr Value:
51	0.2924
52	0.2840
53	0.2757
54	0.2676
55	0.2596
56	0.2518
57	0.2441
58	0.2366
59	0.2291
60	0.2218
61	0.2147
62	0.2076
63	0.2007
64	0.1938
65	0.1871
66	0.1805
67	0.1789
68	0.1675
69	0.1612
70	0.1549
71	0.1487
72	0.1427
73	0.1367
74	0.1308
75	0.1249
76	0.1192
77	0.1135
78	0.1079
79	0.1024
80	0.0969
81	0.0915
82	0.0862
83	0.0809
84	0.0757
85	0.0706
86	0.0655
87	0.0605
88	0.0555
89	0.0506
90	0.0458
91	0.0410
92	0.0362
93	0.0315
94	0.0269
95	0.0223
96	0.0177
97	0.0132
98	0.0088
99	0.0044

from the OPPOSITE ARM. Observe the same precautions with the tourniquet as before.

5. Put this blood into the number 2 tubes and invert four or five times.

6. Centrifuge all tubes for thirty minutes at high speed. Read and record the average meniscus levels and the RBC levels.

7. Pool the plasma from the number 1 tubes into a similarly numbered centrifuge tube and recentrifuge for greater clarity of plasma. Do likewise with the number 2 plasma.

8. Put the uncolored plasma (number 1) into an absorption cell. This is put in place of the distilled water and used to standardize the photelometer needle on 100 (in colorimeters using the per cent scale). The colored serum will be read against this serum.

CALCULATION. It is understandable that gross hemolysis may affect the true final values by 5 to 10 per cent (even when read against a blank), but the use of the K value as determined in the calibration will likely completely eliminate this common source of error.

1. Total plasma volume $= \frac{500}{Lr \div K} \times 5 \times$ dilution factor.*

2. Total blood volume $= \frac{\text{plasma volume} \times 100}{100 - \text{hematocrit}}$.

3. Red cell mass = blood volume — plasma volume.

4. Total plasma proteins = plasma proteins (gms. %) $\times \frac{\text{plasma volume}}{100}$.

5. Total circulating hemoglobin = hemoglobin (gms. %) $\times \frac{\text{blood volume}}{100}$.

CALIBRATION. The 620 mu filter will be used on the photoelectric colorimeter. (This is the red filter commonly used for determination of serum cholesterols and serum phosphorus.)

A. The Evans Blue dye (T-1824) may be obtained from the William R. Warner & Co., New York, N. Y. (This dye is labeled "For investigational use" and is limited to experimental use under Federal Law; it may be obtained by the Medical Staff using specified forms.)

One should obtain sufficient dye to last slightly over one year. This will make the calibration now being planned accurate for that interval of time.

The dye is 5 per cent, and contains 500 mg. per 100 cc.

B. Dilute the dye 1 cc. up to 50 cc. with distilled water. The dilute solution will contain 10 mg. per 100 cc.

C. Prepare the following standards with the dilute dye:

* See Footnote, Page 319.

TUBES	Dil. Dye	Water	Plasma	Final Dil.	Equiv. in Blood	Mg./100 cc.
1.....	1.0 cc.	None	9 cc.	1/500	2500 cc.	1 mg.
2.....	0.9 cc.	0.1 cc.	9 cc.	1/555.6	2778 cc.	0.9 mg.
3.....	0.8 cc.	0.2 cc.	9 cc.	1/625	3125 cc.	0.8 mg.
4.....	0.7 cc.	0.3 cc.	9 cc.	1/714.3	3571 cc.	0.7 mg.
5.....	0.6 cc.	0.4 cc.	9 cc.	1/833.3	4166 cc.	0.6 mg.
6.....	0.5 cc.	0.5 cc.	9 cc	1/1000	5000 cc.	0.5 mg.

Prepare a plasma BLANK using 9 cc. of plasma and 1 cc. of water. This will be used in place of the distilled water in the photoelectric colorimeter, and the above standards read against it. Record the readings below:

STANDARDS	Mg./100 cc.	Meter Readings	Lr Values	K Values
1.....	1 mg.	.....	.....	.....
2.....	0.9 mg.	.....	.....	.....
3.....	0.8 mg.	.....	.....	.....
4.....	0.7 mg.	.....	.....	.....
5.....	0.6 mg.	.....	.....	.....
6.....	0.5 mg.	.....	.....	.....

Note: The meter readings can be converted to the Lr values by consulting the table printed elsewhere in this paper. This table is for use with those colorimeters using a per cent meter scale: those using logarithmic scales give the Lr values direct on the scale. (The Lr value is the logarith of 100 minus the logarith readings of the unknown.)

To determine the K values from the above data:

1. Divide the Lr values of each standard by its mg./100 cc.
2. Average the K values of all the standards and use this average as the K in the above calculation formula.

REPORTING THE RESULTS. The results given by this test may be compared with estimated normals computed individually for each patient. This is more accurately and consistently done on the basis of a per kilogram basis. YOU MUST USE THE NORMAL HEALTH WEIGHT OF THE PATIENT. IF THE PATIENT IS UNUSUALLY THIN OR OBESE IN HEALHY STATES, IT WILL BE MORE ACCURATE TO CONSULT THE WEIGHT CHARTS BASED ON AGE, SEX AND HEIGHT (these may be on charts in the laboratory or in nutrition textbooks.)

The normal values given by Gregerson are used in this laboratory; they are 45 cc./Kg. and 85 cc./Kg., total plasma and blood volumes respectively. If a patient, for illustration purposes, weighs 150 lbs., the kilogram weight would be 150 divided by 2.2, or 68.2 Kg. body weight.

We will suppose the same patient was found to have 3000 cc. of plasma volume and 4797 cc. of total blood volume. The estimated normals for this patient would be 45 multiplied by 68.2, or 3069 cc. of plasma volume. The estimated blood volume normals would be 85 multiplied by 68.2, or 5797 cc. The results

should be reported thus (Beling et al recommended a report similar to the following):

	Total Blood:	Total Plasma:
Estimated Normal Volume;	5797 cc.	3069 cc.
Actual Volume (By Test):	4797	3000
Deficit:	1000	9.

The deficit column shows the actual difference found between the estimated normals and the actual volumes as given by the results of the test. It can be clearly seen that the deficit columns will show the actual amounts of blood and/or plasma needed by the patient; the patient as here shown requires 1000 cc. of whole blood to restore his normal volume of total blood volume.

CONCLUSION. 1. A complete technique is given with precautions. This test is easily performed after the initial calibration is completed in the laboratory.

The calibration method presented is based on the work of O. H. Gaebler. The use of the K value in the formula, as given herein, will read turbid, slightly hemolysed and otherwise unsatisfactory plasma.

2. A method for presentation of results, as advocated by Beling et al, is shown which will indicate directly the actual amounts of blood and/or plasma needed by the patients.

The results rendered the surgeon or physician will help to eliminate shock conditions in major surgery cases, both preoperatively and postoperatively. Also, treatment of acute burns and accident cases will be benefited by these results.

3. Circulating blood volumes are a useful addition to laboratories operating blood banks.

4. This complete technique is mainly a correlation and compilation of several, with a unique calibration method added to it.

The accuracy of this determination as given here is from minus 3% to plus 3%, which compares favourably with the minus 2% to plus 4% values of Gregerson's method.

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THE SEROLOGY OF SYPHILIS WITH SPECIAL REFERENCE TO CARDIOLIPIN AND KOLMER ANTIGENS

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Eight years have now elapsed since Pangborn,[1] by elaborate technical methods, isolated a complex phosphatidic acid from alcoholic extracts of beef heart designated as cardiolipin. This lipid alone was found too low in sensitivity for use as antigen in the conduct of complement fixation and flocculation tests for syphilis. Sensitivity, however, was increased by the addition of purified lecithin from beef heart and, in some methods by the addition of cholesterol as well. The chemical purity of these three lipids and the quantities in which they are concentrated in the final antigen influence both the sensitivity and specifically of serologic reactions. Consequently the optimum amounts of each to employ in the complement fixation and flocculation tests for syphilis vary according to the test or tests employed. In the Kolmer complement fixation test a combination of 0.03 per cent cardiolipin, 0.05 per cent lecithin and 0.6 per cent cholesterol possesses a high degree of sensitivity,[2] although other combinations have been found satisfactory, like 0.03-0.05-0.3 and 0.0175-0.0875-0.3.[3, 4]

The original Kolmer antigen, a cholesterolized and lecithinized alcoholic extract of beef heart, was described in 1922.[5] An alternate method was described in 1935[6] but this antigen was found to give nonspecific reactions with the complement serums of some guinea pigs and prezone reactions with the spinal fluids of some human beings. Under the circumstances a third change in the technic of preparation was described in 1948, designated as *improved* Kolmer antigen.[7] With this antigen it has been found unnecessary to pre-test complement and unnecessary to use egg albumin for the prevention of prezone reactions in tests with spinal fluids.

Probably the sensitivity and specificity of present day complement fixation and flocculation tests can best be expressed according to the results of recent Annual National Serologic Evaluation Surveys since various author serologists and state laboratories have employed cardiolopin and standard antigens.

In the 1944 survey Harris and Portnoy[3] found that a cardiolipin

antigen (0.03-0.05-0.3) had a sensitivity rating of 83.2 per cent and a Kolmer antigen a rating of 83.6 per cent in Kolmer complement fixation tests conducted with the serums of syphilitic donors; in my laboratory Kolmer tests conducted with regular antigen had a rating of 85.5 per cent. In the 1946 survey, Kolmer tests conducted in my laboratory had a sensitivity rating of 88.4 per cent, and the New York State Department of Health Laboratory a rating of 85.0 per cent in a flocculation test conducted with cardiolipin antigen. In the 1947 survey, the Kolmer test conducted in my laboratory had a sensitivity rating of 83.7 per cent while a VDRL slide flocculation test conducted with cardiolipin antigen had a rating of 85 per cent and the New York State Department of Health Laboratory a rating of 75.0 per cent in a flocculation test conducted with cardiolipin antigen.

The results observed in the 1949 survey are shown in the accompanying table. I am presenting these not only because it is reasonably expected that the various tests were conducted in the laboratories of author serologists with scrupulous attention to all technical details, but because this was the first survey in which cardiolipin antigens and *improved* Kolmer antigen were subjected to an extensive comparison with the antigens of various author serologists.

It will be observed that the sensitivity ratings of complement fixation tests with the sera of 237 treated and untreated syphilitic donors varied from 60.1 per cent (Eagle) to 76.5 per cent (Kolmer) while the flocculation tests varied from 65.7 per cent (Hinton) to 75.0 per cent (Mazzini) employing the respective author antigens. With cardiolipin antigens the sensitivity rating of the Kolmer complement fixation test was 78.6 per cent while varying from 69.4 (VDRL) to 76.6 per cent (Kline diagnostic) in four flocculation tests. It will be observed, therefore, that cardiolipin antigen gave 2.1 per cent more positive reactions in my complement fixation test, 7.8 per cent more positive reactions in the Hinton test, 4.0 per cent more in the Kahn standard and 10.2 per cent more in the Kline diagnostic tests. While these comparative results do not justify a final decision on the comparative sensitivity of author and cardiolipin antigens in complement fixation and flocculation tests, they probably indicate that the latter antigens are somewhat more sensitive and especially in flocculation tests.

As previously stated one advantage of cardiolipin antigen is the fact that it is chemically reproducible But while the various cardiolipin antigens were supplied author serologists and state laboratories by a central laboratory, it will be observed in the table that the variations in sensitivity ratings in state laboratories employing the Kahn, Kline and Kolmer tests were practically just as great with cardiolipin antigens as observed in tests em-

ploying standard antigens. In other words, it appears that the chemical reproducibility of cardiolipin antigen has not materially increased the uniformity of positive and doubtful reactions in various laboratories testing the same syphilitic serums.

Andujar, Anderson and Mazurek[8] have reported that Kolmer complement fixation tests conducted with 3067 syphilitic serums and the former Kolmer antigen gave 89.7 per cent and cardiolipin antigen 93.4 per cent positive and doubtful reactions; in tests conducted with 369 non syphilitic serums the Kolmer antigen gave 98.1 per cent and the cardiolipin antigen 94.6 per cent negative reactions. In other words, the Kolmer antigen employed was less sensitive but more specific than the cardiolipin antigen.

Bohls and Shaw[9] have reported that Kolmer complement fixation tests conducted with cardiolipin antigen gave more sensitive reactions than the former Kolmer antigen in testing icteric low-titered syphilitic serums. With 592 serums (syphilitic and non-syphilitic) Kolmer and Lynch[10] observed positive reactions in 36.1 per cent with improved Kolmer antigen and in 37.3 per cent with cardiolipin antigen (0.03-0.05-0.6); in other words the latter gave 1.2 per cent more positive reactions with syphilitic serums since both antigens gave negative reactions with all serums of presumably nonsyphilitic individuals. Giordano, Frost, and Higginbotham[11] have also reported that the sensitivity and specificity of improved Kolmer antigen compares favorably with cardiolipin antigen in Kolmer complement fixation tests while McDearman and Cottrell[12] state that cardiolipin antigen increases the sensitivity of Kolmer complement fixation reactions to some extent with no loss in specificity.

In so far as the Kolmer complement fixation test with normal serums and spinal fluids is concerned, both cardiolipin and Kolmer antigens possess the same high degree of specificity although in some of the flocculation tests cardiolipin antigen apparently shows fewer false positive reactions than standard antigens. But cardiolipin antigen has not solved the problem of biologic nonspecific or falsely positive reactions in leprosy, malaria and other diseases although it is generally agreed that cardiolipin antigen yields fewer false positive reactions in malaria. Stout[13] has reported that cardiolipin antigen gave 34 per cent and Kolmer antigen 19 per cent positive reactions in malaria while Andujar and his colleagues[8] have observed 20 per cent positive reactions with cardiolipin antigen and 40 per cent with Kolmer antigen in this disease. Levine and his associates,[14] however, have reported that while Eagle, Hinton, Kahn, Kline and Mazzini antigens have given 8 to 24 per cent false positive reactions in malaria, cardiolipin antigen has given only about 1 per cent reactions, which is in confirmation of the previously recorded observations of Rein and Kent.[15]

Comparative Sensitivity and Specificity of Cardiolipin and Standard Antigens in the 1948 National Serologic Survey

TESTS	Antigens	CONTROL LABORATORIES*		STATE LABORATORIES	
		†Sensitivity	‡Specificity	†Sensitivity	‡Specificity
Eagle complement fixation	Eagle	60.1	100.0	50.0-67.9	99.7-100.0
Eagle flocculation	Eagle	69.7	100.0	65.7-69.4	99.7-100.0
Hinton flocculation	Cardiolipin	73.5	100.0		
Hinton flocculation	Hinton	63.7	100.0	42.9-78.4	95.7-100.0
Kahn standard	Cardiolipin	70.2	100.0	49.0-61.7	100.0
Kahn standard	Kahn	66.2	100.0	54.1-71.5	98.2-100.0
Kline diagnostic	Cardiolipin	76.6	100.0	71.2-85.3	99.0-100.0
Kline diagnostic	Kline	66.4	100.0	59.6-79.1	97.1-100.0
Kolmer simplified	Cardiolipin	78.6	100.0	73.3-85.6	98.0-100.0
Kolmer simplified	Kolmer	74.4	100.0	58.3-77.9	99.7-100.0
Kolmer quantitative	Kolmer	76.5	100.0		
Mazzini	Mazzini	75.0	99.3	69.3-85.8	97.9-100.0
V.D.R.L.	Cardiolipin	69.4	100.0	68.6-81.4	99.4-100.0

* Tests conducted in the laboratories of the respective author serologists.
† Based on tests with the sera of 237 treated and untreated syphilitic donors.
‡ Based on tests with the sera of 141 presumably nonsyphilitic donors.

In leprosy, on the other hand, cardiolipin antigen gives about the same number of false positive reactions as other antigens in flocculation tests;[16] whether or not this is true in Kolmer complement fixation tests employing the improved Kolmer antigen cannot be stated.

However, the hope that cardiolipin antigen might permit the standardization of serologic testing for syphilis and the abandonment of the confusing claims for the superiority of one or another of the standard antigens has not as yet been realized. As Mahoney[17] has stated editorially:

"Six years ago cardiolipin, as a new phospholipin component of antigen for serologic tests for syphilis, was announced. Clinicians and serologists welcomed this announcement for they hoped that this new substance would be the long-sought-for key to the simplification or, at least, to the clarification of the serology of syphilis. They thought that simplification would be achieved by the adoption of a few universally accepted procedures which would utilize cardiolipin. Thus, the innumerable procedures which used the lipoidal antigens would be eliminated.

"But six years of experience shows that cardiolipin has neither simplified nor clarified the serology of syphilis. Cardiolipin antigens have been adapted to the technics which formerly used lipoidal antigens. The number of technical methods has increased rather than decreased. These new methods are being used by ever increasing numbers of laboratories.

"In the testing of large numbers of specimens from syphilitic individuals, the performance of cardiolipin antigens has been comparable to that of the older antigens; but as yet there has not been sufficient testing of material from diverse nonsyphilitic

individuals to warrant any conclusion concerning the specificity of the modified procedures. Final appraisal of cardiolipin can be achieved only when it has been applied to a significant volume of authenticated, clinically diverse testing material. Perhaps this appraisal will be achieved from the analysis of the mass of data accumulating from the extensive testing now in progress. If not, then the measure of specific reliability must be determined by the more cumbersome procedure of original method evaluation studies.

"To put it simply, although laboratory observations justify much optimism concerning the future of cardiolipin in serologic tests for syphilis, it has not been demonstrated that its use can remove any responsibility from the clinician who interprets the results of laboratory procedures in behalf of the patient for whom the tests are performed."

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GENTIAN VIOLET MEDIUM AS A SCREEN TEST FOR IDENTIFYING THE CANDIDAS

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The identification of the yeastlike fungi is relatively difficult and frequently confusing. Cultural characteristics, unless observed under the most rigidly controlled conditions, are not consistent, and the identification of an organism may be delayed for weeks.

The yeastlike organisms most commonly encountered in the laboratory include the genera Candida, Cryptococcus and Saccharomyces. With the exception of *Cryptococcus neoformans,* the cryptococci and saccharomyces species are of minor importance in medical mycology. The yeastlike organisms produce smooth, raised, usually glistening and slightly mucoid colonies with a yeasty odor. Stains disclose oval, round, or elongated yeast cells, usually budding, with or without mycelia which are septate and nonaerial. Identification necessitates determining the presence and type of mycelium, sugar fermentations, the presence or absence of ascospores, and specific colony appearance.

The clinician is primarily interested in *C. albicans* as the etiological agent of mycotic vulvovaginitis, of bronchial or pulmonary infections simulating atypical tuberculosis and of white patches on the oral mucosa, or eczematous patches on the smooth skin. *Cryptococcus neoformans* is usually diagnosed from a direct India ink spinal fluid or sputum preparation. The confusion accompanying culture identification of the yeasts consequently lies in differentiating the candida species and in ruling out the nonpathogenic cryptococci and saccharomyces which assume importance only because they so closely resemble the candidas.

Because of conflicting cultural reactions attributed to the candidas, a multiplicity of names has been used to designate the same organisms. Since the classification by Stovall and Bubolz[1] and more recently by Jones and Martin[2] and Martin, Jones, Yao and Lee,[3] few valid candida species are now recognized.

Our laboratory has had the opportunity of identifying certain

of these species from a series of vaginal swab cultures taken over a period of 16 months. Yeastlike organisms were found in 53 per cent of the cultures from a group of 155 pregnant and non-pregnant women who complained of vulvovaginal itching. Fifty of the cultures were classified according to species by corn meal agar growth, sugar fermentations, and blood agar colony morphology. The species and percentage occurrence of each are given in Table 1.

TABLE 1

Strains Isolated from Vaginal Tracts of Pregnant and Non-Pregnant Women

	Incidence	Percent
Candida albicans	35	70
Candida stellatoidea	1	2
Candida parakrusei	5	10
Candida Krusei	3	6
Cryptococcus sp.	4	8
Saccharomyces sp.	2	4
Total	50	100

Our routine upon receiving vaginal swabs was to streak dextrose agar pH 5.6 pour plates with the suspected material. The low pH of the medium inhibited many bacteria commonly found on the vaginal mucosa. Although certain bacteria, i.e., proteus, aerobacter and pseudomonas, were less readily inhibited, yeastlike colonies were discernible in mixed cultures. Such colonies were readily observed due to their raised, white usually glistening appearance and their characteristic yeasty odor. Occasionally, more than one type of yeast colony developed from an individual swab culture. Pure cultures of a yeast were frequently encountered.

A methylene blue stain of any suspected colony confirmed the presence of yeast cells. The routine which was followed for the identification of the candida species, the cryptococci, and the saccharomyces and points in technique to be emphasized are as follows:

1. Growth from the original culture must be transferred to Sabouraud's dextrose broth. In 48 hours, bubbly surface growth indicates *C. tropicalis* or a saccharomyces. A pellicle and collar extending 7-10 mm. up the sides of the broth tube is characteristic of *C. Krusei* and no surface growth indicates one of the other candida species or a cryptococcus.

2. Dextrose blood extract agar plates are streaked from Sabouraud's broth cultures and incubated at 37° C. for 10 days. *C. albicans* colonies are cream colored, smooth and 5-10 mm. in diameter. *C. stellatoidea* colonies are smooth and cream colored, similar in size to *C. albicans*, but have protruding arms and they are referred to as "star shaped." *C. parakrusei* colonies are small,

about 1-2 mm. in diameter, pearly white, raised and glistening. *C. Krusei* colonies vary considerably in size, are grey, dull and flat, and usually have a dry crusty appearance. *C. tropicalis* and *Saccharomyces* sp. are white and raised and about 4-7 mm. in diameter. *C. tropicalis* develops a definite mycelial fringe which can be seen microscopically surrounding the colony. Cryptococcus colonies develop with difficulty, not exceeding 1 mm. in diameter.

3. Transfers for "pure" strains from blood agar must be made to sugar free media, preferably plain agar slants, and successive transfers on this medium must be made for three generations.

4. Corn meal agar plates are streaked from the last plain agar transfer by cutting the agar with a straight inoculating needle. Typical mycelial growth is apparent in 2-3 days. *C. albicans* produces mycelium-bearing, ball-like clusters of budding cells and characteristic thick-walled chlamydospores. *C. albicans* is reportedly the only species to form chlamydospores although our strain of *C. stellatoidea* has, under certain conditions, produced chlamydospores. Mycelia arrangement of each of the candidas vary considerably but are specific for each species. Saccharomyces and cryptococci form no mycelia.

5. Carbohydrate broth tubes pH 7.2 with brom thymol blue indicator are inoculated from saline suspensions of the last plain agar transfer. Sucrose, maltose, dextrose and lactose fermentations differentiate the species. All tubes must be vaseline sealed.

6. Carrot plugs must be inoculated to demonstrate asci formation of the saccharomyces.

7. Cryptococci species encountered by us grow poorly on blood agar, form no mycelium, and ferment few sugars.

These procedures indicate the extensive time consumed in species identification of the candida. This is impractical for routine laboratory work. A medium which might serve as a differential agent for these fungi would have many advantages. Littman[4] proposed an oxgall agar selective for fungi which seemed to offer certain advantages for early identification of the candidas. This medium, in which crystal violet 1:100,000 is incorporated and to which streptomycin can be added as a bacteriostatic agent, was reported as supporting growth of *C. albicans*. Routine use of Littman's agar failed in our experience to serve for primary isolation of the candidas. Pure cultures of the fungi when heavily inoculated upon this medium did develop but grew very slowly and sparsely.

Gentian violet 1:25,000 and also 1:10,000 have been used successfully in treating *C. albicans* vaginal infection.[5] Tanner and Bollas[6] reported that when gentian violet was incorporated into media in a dilution of 1:80,000, no growth of yeasts appeared on

the dye media, whereas in a dilution of 140,000 only very slow growth of these yeasts appeared in 3-4 days. Further investigation of the use of gentian violet in a suitable medium seemed to offer possible advantage in the isolation and diagnosis of the candidas.

Since the clinician is interested chiefly in the pathogenic species *C. albicans*, we have endeavored to find a gentian violet dye concentration in a medium which might serve to differentiate this species from the others. Dye concentrations of 1:100,000, 1:200,000, 1:400,000 and 1:800,000 in dextrose agar were tested for supporting growth of the candidas. *C. albicans* was entirely inhibited up to 5 days when inoculations were made on the agar with dyes of the first two concentrations. Media with gentian violet 1:400,000 and 1:800,000 inhibited *C. albicans* to some extent and the other species to a much less degree. From observations of growth, colony morphology and pigmentation made daily for 7 days, a medium with a dye concentration somewhere between these two concentrations was decided upon as probably most nearly a satisfactory medium for partial inhibition and for showing outstanding colonial characteristics of the candidas. Dextrose agar with gentian violet 1:600,000 was consequently used for further studies.

Typical cultures of each species of candida available, a saccharomyces and a cryptococcus were inoculated from saline suspensions upon the dye medium of gentian violet 1:600,000 concentration and observations recorded for a two weeks period. Table II shows growth characteristics during this period.

TABLE II

Growth Characteristics of Yeastlike Cultures on Gentian Violet Dextrose Agar

SPECIES	3-4 Days	Growth	Pigment	Colony Morphology	2 Weeks
C. albicans	.1-.5 mm.	Somewhat inhibited, discrete	Lavender	Pyramidal, dry, dull	1-2 mm.
C. stellatoidea	.1-.5 mm.	Somewhat inhibited, discrete	Lavender	Pyramidal, dry, dull	1-2 mm.
C. tropicalis	1-2 mm.	Inhibited	Pale lavender, centers white	Raised, rounded, mycelial fringe evident	4-5 mm.
C. parakrusei	2-3 mm.	Good, confluent	Pale lavender, glistening	Raised, rounded, smooth	5-7 mm.
C. Krusei	1-2 mm.	Good, confluent	Pale lavender, dull, velvety	Very slightly raised, concentric rings with age, may become irregular, mycelial fringe evident	7-10 mm.
Cryptococcus sp.	Minute	Very inhibited	Lavender to purple	Not characteristic	Not over 1 mm.
Saccharomyces sp.	2-3 mm.	Good, confluent	Lavender, glistening	Raised, rounded smooth	5-7 mm.

As a result of these observations, the following routine for studying vaginal swabs was inaugurated.

Plates of Difco dextrose agar were prepared after adjusting to a pH of 5.6 and adding gentian violet 1:600,000. Similar plates of this medium without the dye were also prepared. Sterile wooden applicator swabs were available for use by the clinician in obtaining material from the patients. The exposed swabs were inserted in tubes each containing 1 cc. of sterile broth; these, when received, were streaked upon both plates of agar.

Yeastlike colonies developing within 2-3 days on the plain dextrose agar but concurrently failing to develop in this time on the dye medium were tentatively classified as *C. albicans* or *C. stellatoidea*. At the end of 4 days, small lavender colonies of these two species were usually apparent on the dye medium. Colonies of *C. tropicalis*, *C. Krusei*, *C. parakrusei* and the saccharomyces were suspected when growth on both plates occurred within 2-3 days. *C. parakrusei* and saccharomyces colonies were larger, usually a paler lavender and more mucoid in appearance on the dye medium than were the pyramidal colonies of *C. albicans*. As on the plain dextrose agar, *C. Krusei* colonies assumed a flat, crusty, dry appearance and developed a deeper lavender color in 7-10 days. The presence of yeast colonies on the plain agar, but complete absence of any colonies on the dye medium within 4-5 days, was indicative of a cryptococcus. In 4 days, *C. albicans* and *C. stellatoidea* were easily distinguishable on the dye medium and stains always revealed their presence. Occasionally growth of the fungi was obscured by overgrowth of bacteria, but we always were able to demonstrate the presence of yeast cells by stains within 4 days. The nonpathogenic species were always discernible by gross examination, even when bacterial overgrowth was marked.

Primary growth on gentian violet media of the yeasts cultured from vaginal swabs verified the results obtained from pure culture studies. To confirm the diagnoses tentatively made, each yeast was classified by the systematic method previously employed for our original identification. The use of 1 per cent dextrose blood extract agar differs from the plain blood extract agar used by Martin, Jones, Yao and Lee[3] in promoting more luxuriant growth of the candidas and in showing the striking star formation of *C. stellatoidea* within 2-3 days.

Gentian violet in dextrose agar inhibited the growth of the candidas, but its restraining action on the various species was not the same. This made possible a screening medium which distinguished *C. albicans* and *C. stellatoidea* from the other candida species.

THE PRODUCTION OF VARIOUS SERUM PROTEIN FRACTIONS BY USE OF ION-EXCHANGE RESINS*

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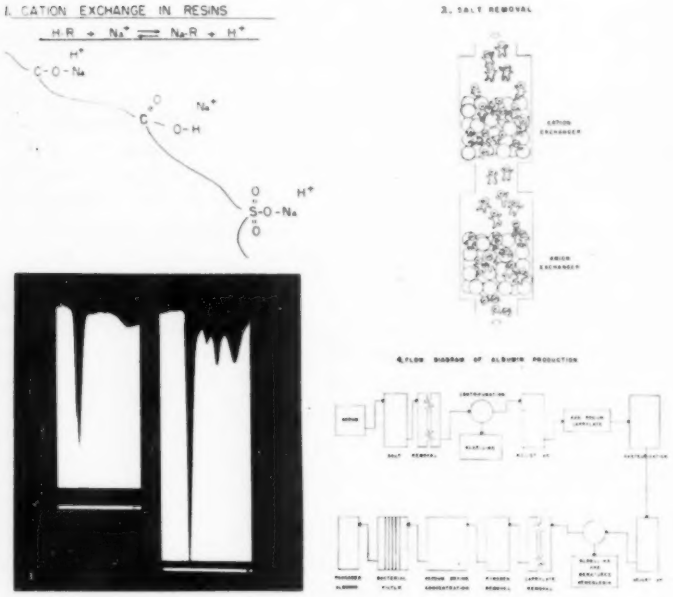
This paper is on the use of ion-exchange resins and their application to the separation of serum proteins. Ion-exchange resins are comparatively new materials in the biological field, but have been used for some time as water softeners in industry and recently in the home. In effect, they are solid ions. The first slide shows diagrammatically how ion-exchange resins work. If you look at a bottle of ion-exchange resin you will see a heterogeneous group of particles which may remind you of coffee grounds, since they are of approximately the same size and appearance. They are comparatively insoluble in water, and when placed in an aqueous solution do not change in appearance, except for a slight swelling at times. On the surface of this inert-appearing material and on the interior structure are chemically active groups. In a typical cation-exchange resin such as the one shown on the slide, these chemically active groups are negatively charged and ionic in nature, acting in exactly the same way as a free negative ion such as the chloride ion, phosphate ion, or acetate ion. In order to keep this substance electrically neutral, it must hold on these active groups some positively charged ion, such as a hydrogen ion or a sodium ion or a calcium ion. If we place some of this resin containing sodium ion in a solution with a high concentration of hydrogen ion, it will give up a large portion of the sodium ions in return for some of the hydrogen ions. If, then, we transfer this resin with a high concentration of hydrogen ions to another solution with a high concentration of sodium ions, it will give back those hydrogen ions to solution and take on sodium ions. In this way it is possible to substitute one ion for another ion in solution without the addition of any solution, without boiling or precipitation, and without otherwise effecting the solution. What can be done with cation-exchange resins in exchanging positive ions or cations can also be done with anion-exchange resins in exchanging anions. Thus, if we have a solution containing chloride ions, we may place an anion-exchange resin saturated with hydroxyl ions into the solution and have the hydroxyl ions substitute themselves into the solution in return for chloride ions which are then taken out by the resin.

Now let us see what can be done by the use of both anion- and cation-exchange resins. (Slide 2). If we have a solution with sodium chloride in it, we can place it with a cation-exchange resin which had been saturated with hydrogen ions. A large portion of the sodium ions will then be taken out of solution,



leaving us a solution of hydrochloric acid. Then, if we place that solution of hydrochloric acid with an anion-exchange resin which had been saturated with hydroxyl ions, the chloride ions will be exchanged for hydroxyl ions, the hydroxyl ions will react with the excess hydrogen ions, giving water, and we will have substi-

tuted water for sodium chloride in the solution. That method can be, and is, used to de-salt solutions. The individual resins can be used over and over again by merely conditioning them before using to the ion you wish to substitute in the solution.

In the case of blood serum proteins, a high hydrogen ion concentration such as would have resulted if the foregoing method had been used would denature or precipitate some of the constituents. But if the two resins, the anion-exchange resin and the cation-exchange resin, are mixed in such a way that the sodium ions and the chloride ions are removed at the same rate the pH will remain fairly constant.

Blood plasma proteins are colloidal particles which do not form true solutions in water, and are held in suspension by internal charges in the solutions. The solubilities of the various globulins and fibrinogen are dependent on the concentration of the ions in the solution. If part of the normally occurring salts are removed from the blood plasma, fibrinogen will precipitate out. If more salts are removed, a gamma globulin will precipitate out. If still more salts are removed beta globulin and finally alpha globulin will precipitate out. So you see that by removing salts with ion-exchange resins it is possible to effect a separation of the proteins of blood plasma. A clean-cut separation will not be entirely obtained at first, but the precipitated fractions can be re-dissolved in an appropriate suspending medium, giving greater and greater purity. The next slide shows an electro phoretic pattern of normal serum on the left. The proteins shown from left to right are gamma globulin, beta globulin, alpha globulin, and albumin. After all of the salts had been removed from this serum and the precipitate centrifuged out, the electrophoretic pattern was that on the right, showing that all of the globulins had been removed, leaving only the albumin.

The next slide shows a diagrammatic arrangement of fractionating the serum into its constituent parts. Here we see the serum go through columns or beds of mixed resins until all except the water-soluble globulins are precipitated and centrifuged out. The salt-free supernatant is then adjusted to a comparatively high pH with an anion-exchange resin, and sodium caprylate is added. The albumin thus thermally protected by caprylate, is pasteurized. After pasteurization the pH is lowered by the use of a cation-exchange resin, and all denatured globulins and hemoglobin are precipitated. Sodium caprylate is then removed by mixed resins, and finally, pyrogens, if present, are removed

by a cation-exchange resin. The pure, but somewhat dilute albumin solution is then concentrated by vacuum drying, filtered through a bacterial filter and finally packaged sterilly.

As a by-product there are the valuable, largely undenatured globulins which can be used in their somewhat crude fractions as they are precipitated, or which may be further purified for specific uses.

COMPARISON OF PLASMA PROTEIN VALUES DETERMINED BY THE SPECIFIC GRAVITY AND THE MICRO-KJELDAHL METHODS

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The saving of time in a laboratory is of great importance, and for this reason, as much as for any other, the clinical chemist has been eager to find a short accurate method for the determination of plasma or serum protein. The specific gravity of plasma or serum has been used as the basis of several procedures proposed for this purpose. This paper describes briefly the more commonly used specific gravity methods and considers the accuracy of results obtained by some of them.

By determining the specific gravity of plasma gravimetrically and at the same time analyzing the plasma for protein by the Kjeldahl procedure, Moore and Van Slyke[13] were able to draw a straight line curve and from this to set up a formula by which the protein content of the plasma can be calculated from the specific gravity. This formula, (??) Grams protein per 100 cc. = 363 (specific gravity—1.007), has been widely used. Several similar formulas for calculating total protein or albumin of human serum or plasma have been suggested by other investigators.[2,4,12]

Besides gravimetric procedures for the determination of specific gravity of plasma, several other methods have been devised. Among them are the falling drop technique presented by Barbour and Hamilton[3] in 1926, the gradient tube method first described by Linderstrom-Lang et al,[8,10] the glass bead method of Bing,[5] and the copper sulfate method reported by Phillips et al[16] in 1943. A brief description of these methods follows.

Barbour and Hamilton supported a tube 50 cm. in length and 7.5 mm. in diameter, filled with xylene and bromobenzene mixture, in a glass cylinder filled with water. By delivering drops of standard solutions of K_2SO_4 and a drop of plasma into the xylene-bromobenzene mixture and noting the time taken for

*Read before ASMT Convention, June, 1949, Roanoke, Va.

each drop to fall a distance of 30 cm. between marks on the inside cylinder, the specific gravity was determined. Both temperature and the size of the drops must be controlled. Kagan[9a, 9b] in 1938 used a mixture of methyl salicylate and mineral oil for the test solution and was able to use a shorter cylinder 16 cm. in length. Földes[6] in 1941 described a procedure which utilized a round glass vessel with a long calibrated neck. The round part of the vessel was filled with a mixture of chloroform and benzene in definite proportions. A drop of plasma was introduced into the mixture. If the plasma was heavier than the mixture, more chloroform was added until the drop remained suspended; if the plasma was lighter than the mixture, more benzene was added. When the drop remained suspended, the specific gravity of the mixture, and hence of the plasma, was read from the graduations on the neck of the flask.

To prepare a gradient tube similar to the kind described by Linderstrom-Lang, Lowry et al[11, 12] used a cylinder within a cylinder. The smaller cylinder was filled to its center graduation with a mixture of bromobenzene and kerosene with a specific gravity of 1.07, which is greater than that of plasma. On top of this was layered an equal amount of a mixture of the same two chemicals with a specific gravity of 0.99, which is less than that of plasma. The two mixtures of different densities were mixed by moving a copper stirrer up and down for a period of minutes, beginning at the center graduation and gradually increasing the distance through which the stirrer was moved up and down. To determine the specific gravity of a body fluid, drops of standard solutions of sodium chloride or potassium sulfate with specific gravities within the range 1.07 to 0.99 were delivered into the inside cylinder, and each drop took a position where the density of the surrounding medium was exactly equal to the density of the drop. The specific gravity of the body fluid was determined by noting the position of the drop of fluid introduced into the cylinder in relation to the position of the drops of standard solution. The gradient tube must be undisturbed and kept away from direct sunlight and other sources of heat. In a simplification of the gradient tube method, Ponder[17] used a single graduated cylinder.

Bing in his method used one glass bead with a specific gravity equal to that of plasma which has a protein concentration of 3.0 gm. per cent. For the test he placed the bead in 3 cc. of plasma and then added enough 0.9 per cent NaCl solution to cause the bead to sink from the surface of the solution into the diluted plasma and to remain suspended there. The specific gravity of the plasma was then calculated from the amount of NaCl solution used. Simeone and Sarris[19] varied this method and used many glass beads of graded specific gravities; by noting which

bead remained suspended in the plasma for 30 seconds without rising or falling, they determined the specific gravity of the fluid. Mortensen[14] made use of a 1 or 2 cc. pipette and two glass beads of known specific gravity. The beads were placed in the pipette, and constrictions were made at each end of the pipette so that the beads were not removable. In determining specific gravity, the pipette was filled with plasma and its ends were closed with a rubber band. When the pipette was placed in a vertical position, the beads took their respective positions, the lighter one migrating to the upper constriction of the pipette and the heavier one going to the lower constriction. The pipette was inverted through 180°, and the beads moved toward each other. The point on the pipette at which they met was noted. From the known data, the specific gravity was then calculated.

In a method devised by Phillips et al[16] in Van Slyke's laboratory, a series of tubes containing copper sulfate solutions of graded specific gravities were prepared. When a drop of plasma is delivered into a solution of copper sulfate, the protein of the plasma precipitates as copper proteinate. If the specific gravity of the solution in the tube is the same as that of the plasma, the drop of plasma will sink only a few cm. into the solution and then will remain suspended for 15 to 20 seconds without rising or falling. After about 50 tests have been done with 50 cc. of diluted copper sulfate solution, the solution should be replaced. The stock solution of copper sulfate must be prepared accurately. It keeps indefinitely.

Each of the different specific gravity methods which have been mentioned and briefly described requires simple equipment. None except the gravimetric procedure of Moore and Van Slyke necessitates the use of an analytical balance, except for the preparation of solutions. All have been reported to give results which agree, at least roughly, with values obtained by the micro- or macro-Kjeldahl determination. Since less equipment and less time are required when analyses are done by specific gravity methods than by Kjeldahl procedure, it is important that we know to what extent results by specific gravity and Kjeldahl procedures agree.

In our laboratories* plasma protein was determined by either the falling drop[3] or the copper sulfate[16] method on 219 heparinized plasmas from normal infants and normal and ill children and adults. These plasmas were also analyzed by the micro-Kjeldahl procedure of Hill and Trevorrow.[7] On 93 of the plasmas, analyses were made by the micro-Kjeldahl and the falling drop procedures; in the analyses on the remaining 126

*Part of the data used is from the Department of Human Growth, University of Colorado Medical Center, Denver, Colorado.

plasmas, we compared the micro-Kjeldahl and the copper sulfate procedures. Results are shown in the accompanying table.

On 71 per cent of the 219 plasmas analyzed, results by specific gravity and Kjeldahl agreed within ±0.4 gm. protein per 100 cc. of plasma. On three plasmas compared by copper sulfate and Kjeldahl, the difference in results was as great as ±0.9 gm. per 100 cc.; on one plasma the difference was 1.34 gm. The greatest difference found by comparison of falling drop with Kjeldahl was between 0.7 and 0.8 gm. per cent. A total of 97 plasmas were compared by these two methods, and only one plasma showed a variation as great as 0.7 gm. per cent by these two methods.

Other investigators have reported similar variations in results comparing plasma or serum protein values by the Kjeldahl procedure and specific gravity methods. Kagan[9b] analyzed 107 sera by the macro-Kjeldahl procedure and by his modification of Barbour and Hamilton's falling drop method. He claims a mean deviation of ±0.16 gm. per cent and a maximum deviation of ±0.48 per cent in analyses on 107 sera; on 122 plasmas he reports a mean deviation of ±0.23 gm. per cent and a maximum deviation of ±0.59. In a comparison of results with the gradient tube and by Kjeldahl on sera from 240 patients with a variety of diseases but "without obvious liver damage," Lowry and Hunter report a difference of 0.4 gm. per cent or more of protein in 5 per cent of the sera analyzed, and a maximum difference of 0.7 gm. per cent. Bing compared his glass bead method with the Kjeldahl method on 100 sera; he reports that 95 per cent of the values agreed within ±0.42 gm. per cent. Bing also reports, however, that one patient with anuria and a blood urea value of 444 mg. per cent showed 7.35 gm. per cent serum protein by the Kjeldahl procedure and 8.41 gm. per cent by the glass bead method. Schousboe[18] did Kjeldahl and gravimetric specific gravity determinations and found a maximum variation of 0.32 per cent protein on the sera of 20 persons—3 healthy individuals and 17 with random pathological conditions; however, on a patient with amyloid degeneration of the kidney and with an accompanying blood non-protein nitrogen of 168 mg. per cent, the values by the two methods differed by 0.8 gm. per cent.

Adams and Ballou[1] studied a series of 128 burn cases by the copper sulfate and Kjeldahl procedures. Most of the patients had been hospitalized over a long period of time. They used serum for their analyses, which they feel gives more accurate results than plasma. In 35 per cent of the cases, the results agreed within 0.2 gm. per cent. In 44 per cent of the cases, agreement was within 0.3 gm. per cent. A total of 21 comparative values, however, differed by more than 1.0 gm. per cent, with five values differing up to 1.4 per cent. These workers feel that

TABLE I

Comparison of Plasma Protein Values by Specific Gravity and Micro-Kjeldahl Methods on 219 Normal and Pathological Plasmas

Negative and Positive Figures Indicate Micro-Kjeldahl Values Lower and Higher, respectively, than Specific Gravity Values.

Difference Between Specific Gravity and Micro-Kjeldahl	Number of Cases	Difference Between Specific Gravity and Micro-Kjeldahl	Number of Cases
—1.4 Gm. to —1.3 Gm.	1	—0.2 Gm. to —0.1 Gm.	16
—1.3 Gm. to —1.2 Gm.	0	—0.1 Gm. to 0 Gm.	25
—1.2 Gm. to —1.1 Gm.	0	0 Gm. to +0.1 Gm.	27
—1.1 Gm. to —1.0 Gm.	0	+0.1 Gm. to +0.2 Gm.	30
—1.0 Gm. to —0.9 Gm.	2	+0.2 Gm. to +0.3 Gm.	18
—0.9 Gm. to —0.8 Gm.	5	+0.3 Gm. to +0.4 Gm.	14
—0.8 Gm. to —0.7 Gm.	1	+0.4 Gm. to +0.5 Gm.	15
—0.7 Gm. to —0.6 Gm.	5	+0.5 Gm. to +0.6 Gm.	12
—0.6 Gm. to —0.5 Gm.	5	+0.6 Gm. to +0.7 Gm.	8
—0.5 Gm. to —0.4 Gm.	4	+0.7 Gm. to +0.8 Gm.	4
—0.4 Gm. to —0.3 Gm.	14	+0.8 Gm. to +0.9 Gm.	0
—0.3 Gm. to —0.2 Gm.	12	+0.9 Gm. to +1.0 Gm.	1

the correlation betwen specific gravity and the protein content of serum is too low to make it possible to place much dependence upon specific gravity determinations for plasma protein. Atchley et al[2] also found poor agreement between copper sulfate and Kjeldahl analyses.

Since constituents of serum or plasma other than protein contribute to its specific gravity, an exact correlation between protein values calculated from specific gravity measurements and from Kjeldahl results cannot be expected. Lowry and Hunter state that apparent serum protein is changed 0.1 gm. per cent by doubling of the normal serum lipids, blood glucose or non-protein nitrogen. According to Zozaya,[20] the specific gravity of serum depends partly on the relationship between bound water and free water in the serum, and bound water depends on the kind and percentage of the protein fraction, euglobulin having a higher specific gravity per unit concentration than albumin. The work of Nugent and Towle,[15] who separated the albumin and the globulin of beef blood and then prepared synthetic solutions with various proportions of these two proteins, indicates that albumin and globulin have the same specific gravity effects.

From our results and from reports of other investigators, it must be concluded that correlation between the plasma or serum protein values calculated from specific gravity measurements and the results obtained from analysis by micro- or macro-Kjeldahl procedure is often very poor; and that specific gravity should not be used in determining plasma proteins without full appreciation of the errors inherent in such methods.

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A COMPARISON OF SEVERAL NEWER METHODS OF PLASMA-PROTEIN DETERMINATIONS

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Plasma proteins are complex colloids which constitute over eighty-five percent of the total solids of blood plasma, either in the form of simple proteins or linked to other compounds. Investigations have shown that both qualitative and quantitative changes occur in many diseases, therefore we can readily perceive the necessity for accurate and simple procedures to determine the total protein and the content of the various protein fractions. Consequently, numerous methods have been evolved, for the complexity of the protein molecule has made this a difficult task and we must now evaluate each of these methods for accuracy, suitability for clinical use and reliability of results obtained.

A. Total Proteins

For the determination of total protein the most accurate method is of course, the gravimetric determination, but as is true of all gravimetric procedures it also, is tedious and time consuming. Hence, it long has been established that the Kjeldahl method of nitrogen determination is the method of choice although it too, may introduce error in employing the figure 6.25 for the calculation of protein from the nitrogen content. The difficulties of time and technique has excluded this from practical routine use and in its place the Kingsley Biuret reaction is now widely employed. Weichselbaum[1] in 1946 modified the Biuret reaction and he describes the use of a "one-piece" reagent containing the copper salt of sodium-potassium-tartrate with the addition of potassium iodide to prevent autoreduction. This reagent can be added directly to proteins and protein dilutions to give a relatively permanent and optically clear solution suitable for photocolorimetric estimation. Weichselbaum[1] states that his procedure overcomes the obstacles of turbidity and limited stability of reagents prevalent in the Kingsley Method; and further states that a comparison of his method with the macro-Kjeldahl proved it "sufficient for all ordinary clinical purposes."

B. Protein Fractionations

Nearly all protein fractionations are based on the ability of salt solution to precipitate plasma proteins at specified concentrations of the salt. With this principle in mind we can realize

at once what a difficult chore confronts us for protein molecules vary in size, shape, chemical reactivity and solubility; these properties tend to render fractions inhomogeneous; due to imperfect precipitation at the specified concentrations and thus cause an "overlapping" of the precipitating fractions. The "overlapping of the various protein fractions must be stressed for this is the obstacle which has been the point of controversy among numerous investigators. Despite these disadvantages the simplicity of the salting-out methods has made them preferable for clinical use.

(1) NOMENCLATURE

Sorensson[2] in his study of the behaviour of serum globulins divided them into two general types according to solubility in salt-free water. The globulin insoluble in salt-free water he designated Euglobulin and the globulin soluble in salt-free water he designated Pseudoglobulin. Although this distinction is not well defined the nomenclature is useful and many methods employ these terms. In electrophoresis the greek letters alpha, beta, gamma are used to show consecutive fractions where the alpha globulin is next to the albumin.

(2) SALTING-OUT METHODS

Howe[3] in 1921 established the basis for numerous salting-out procedures when he employed Sodium Sulphate as the globulin precipitant and the majority of recorded data shows the use of this method accepted as Howe proposed for about twenty-five

Table I

	HOWE	MAJOOR		MILNE	
Protein Fractions	Pptd by gms Na_2SO_4/100 ml at 37° C.	Pptd by gms $N^1Na_2SO_4$/100 ml at 37° C.	Electro Phoresis	Pptd by gms Na_2SO_4/ 100 ml at 37° C.	Electro Phoresis
Euglobulin	13.5	18.5	γ	19.6	$\gamma+\beta$
Pseudoglobulin I	17.5	26.8	$\alpha+\beta$	26.8	$\alpha_1+\alpha_2$
Pseudoglobulin II	21.5				
Albumin	.....	.....	Alb.	.....	Alb.

years. Majoor[4] and Milne[5] criticized Howe's separation and improved his technique with extensive comparisons. The foregoing Table I shows the results obtained by the three investigators. By increasing the concentrations of salt the globulin fractions will precipitate, the highest concentrations 26.8 precipitates all the globulin fraction and the filtrate of this separation will contain only albumin.

Howe[3] indicated that the precipitates at 13.5 and 21.5 were

definite and that, that of 17.5 was equivocal but Majoor, Milne and other workers were unable to reproduce these curves and when the Howe separations were subjected to electrophoresis they found that the 13.5% sodium sulphate fraction was homogeneous and consisted of the gamma fraction. Only a part of the gamma globulin is precipitated as is confirmed by the Majoor fraction precipitated at 18.5% Sodium Sulphate concentration which is gamma globulin. Furthermore when the filtrate of the 21.5% Howe precipitation was subjected to electrophoresis it still contained some alpha and beta globulin.[6] We can see that if the albumin fraction is higher than it should be and the euglobulin fraction lower, the albumin/globulin ratio would be higher. Peterman, Young & Hogness[7] have stated that the Howe fractionation on the basis of electrophoresis gives results 20-35% higher for normal sera. When higher salt concentrations are used the percentage error is reduced. Again we must constantly bear in mind that these separations are not distinct and exact because in addition to those reasons already mentioned we have the interfering substances of lipids and other substances present in increased amounts as a result of disease.

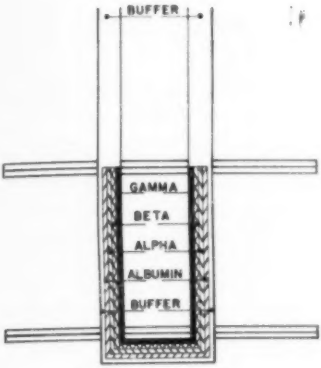
(3) ALCOHOL METHODS

The salting-out precipitations have been supplemented by two other methods, the alcohol method and Cohn's ethanol method. Based on the principle that methanol will precipitate serum proteins at temperatures between 0°-5° C., Pillemer and Hutchinson[8] fractionated proteins by the use of methanol at 42.5% concentrations; a pH 6.7-6.9; ionic strength 0.03; temp. 0° C. Under these conditions they precipitated all the globulin fraction with almost all of the albumin remaining in the solution.

(4) ELECTROPHORESIS

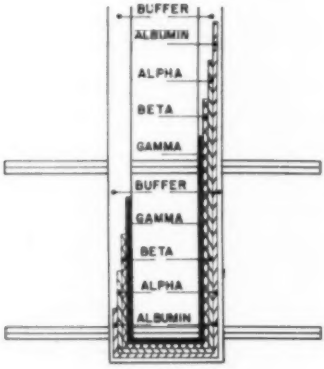
With the introduction of Electrophoresis by Tiselius in 1930 another method to facilitate protein fractionation was initiated. Electrophoresis measures the migration of charged protein molecules in solutions standardized for pH and ionic strength under the influence of an electric current and records these changes photographically. Fig. I & Fig. II will illustrate the principle of this method. In Fig. I the serum which has been previously dialyzed is placed in a u-shaped cell. The proteins are now equally distributed in a buffer medium. When an electrical current is passed through the cell then the proteins will migrate towards the anode. The speed of the protein fractions varies with the size and charge of the particles and the albumin fraction containing the smallest size and the greatest charged molecules will have the greatest speed; therefore this fraction will show the greatest amount of change in migration, and the gamma fraction having

FIGURE 1



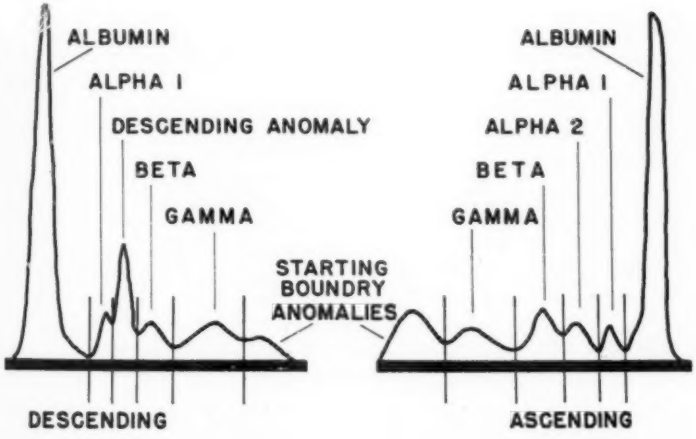
START OF RUN

FIGURE 2



END OF RUN

FIGURE 3



the larger size and the smallest charged molecules will show the least amount of change in migration. The amount of change produced by each globulin fraction is recorded photographically as shown in Fig. III.

Although electrophoresis is of great assistance in the analysis and purification of plasma proteins it too has its disadvantages. The complexity of the apparatus, the length of time for the analysis are the main disadvantages which have limited its use to problem studies as a valuable aid to the separation of the plasma proteins.

C. Results

A comparison of the four methods for total protein determination, demonstrates that sera from both normal and abnormal individuals, the mean result in gms/ml. shows close agreement; the Kingsley Biuret has a greater Standard Deviation and there was a greater margin of error in Standard Error and Precision. A better Standard Deviation and Standard Error is offered by the Weichselbaum Method on the basis of the Gravimetric and Kjeldahl Methods. The Precision of the Weichselbaum Method is also better than the Kingsley Biuret Method. Precision was calculated by the formula $\rho = \sqrt{\frac{\Sigma (x^1 - x^2)^2}{2n}}$ where $x^1 - x^2$ is the difference between duplicates and n equals the number of pairs.

In Table II a series of fourteen patients both normal and abnormal were compared according to Electrophoresis, Milne separation with subsequent Kjeldahl determination of the filtrate, and Biuret by Weichselbaum Method. The total Protein as determined by Electrophoresis is slightly higher because of lipid interference and results show a wider Standard Deviation, between Weichselbaum and Electrophoresis vs. Milne. There is closer agreement between Milne and Electrophoresis although here too we must consider lipid interference.

The preceding results represent comparisons studied at the Pepper Laboratory and Nutritional Service of the University of Pennsylvania Hospital of Philadelphia, under the direction of J. G. Reinhold Ph.D. and J. R. Neefe M.D. This study will be published in detail elsewhere.

Table II

Method	No. Pts.	T.P.	Albumin		Globulin		Eug. ($\beta+\gamma$)		Pseudo (α)	
	Mean	Dev.	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.
Electrophoresis.....	14	7.42	3.22	1.01	4.20	0.914	2.53	0.81	1.63	0.44
Milne : Kjeldahl.....	14	7.10	3.26	0.95	3.84	1.00	2.46	0.83	1.39	0.33
Biuret : Weichsel.....	14	7.13	3.47	1.04	3.66	1.08	2.20	0.92	1.47	0.31

D. Conclusions

We can conclude then that the Weichselbaum Biuret Method using the Milne separation at 19.6 and 26.8 is a practical and adaptable method, although the Gravimetric and Kjeldahl Methods should still be used for standardization purposes. Electrophoresis is of course the standard method of separation but too intricate and too lengthy for routine application.

It is well to bear in mind that the protein fractionation method tested does not provide a pure separation. The methods used, together with the complex state of the plasma proteins can only serve at their best as empirical data. Nevertheless valuable information given by the individual methods employed and offers promising possibilities in elucidating the problem of plasma proteins in health and disease.

Note: Gornall, Bardawill & David have recently published in J. Biol. Chem., of Feb. 1949 a series of studies in which they have modified the Weichselbaum Reagent to contain little or no Potassium Iodide, a lower concentration of Copper and more Alkali. They report excellent results in the use of this reagent.

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EFFICIENCY IN THE LABORATORY

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Efficiency in the laboratory implies speed and accuracy. These can be accomplished in a well organized laboratory in many ways, but only in a well organized laboratory. What is a well organized laboratory? One which has all of the most modern equipment? No, not necessarily. But it is one in which each person in the laboratory knows what is expected of her and performs her duties in a conscientious manner, each contributing her part to the whole. It is a laboratory which puts out the maximum amount of accurate work in the minimum of time.

As in any organization, the head needs assistance. It is impossible for one person to handle every detail in the laboratory,

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unless it is a very small place. The assistant should be permitted to take the responsibility in the department in which she is placed. This holds good even where there is rotation among the technologists. This does not necessarily include the privilege of changing the methods, etc., in the department, but does include supervision of the work.

One way in which to stimulate interest in the laboratory work is to hold a meeting periodically, perhaps every month or six weeks, to discuss any improvements or changes that could or should be made. When the laboratory staff is permitted to offer suggestions or friendly criticism, it helps to create a congenial atmosphere and readily brings about cooperation among the staff members. This greatly increases the total efficiency of the laboratory. Very interesting cases or research problems might be discussed briefly, to inform the staff as to what is being undertaken by its members.

The routine schedule should be flexible enough to permit a certain amount of study and research. These studies should then be written up and presented for publication for the benefit of others. Are we as technologists too selfish, guarding our time and energies too jealously, or are we merely disinterested, neglecting our obligation to contribute our share?

One means of achieving efficiency by accelerating the pace of the technologists is allowing the staff at least five minutes for relaxation or refreshment in the morning and in the afternoon. It is a biological fact that both the mental and the physical powers are quickened by a short rest period.

The laboratory should be regular subscriber to journals and other publications which enable the staff to keep abreast with the times. It would be profitable to have a fund for that particular purpose. In that way the whole laboratory staff will benefit without any one person being overtaxed.

Another little point which we are apt to overlook is this; viz., that we, as medical technologists, are to work with the doctors, not against them. That is the main purpose of our existance as technologists. We are not an independent unit, capable of operating independently of the other branches of the medical profession, as we know from our Code of Ethics. Hence, we must recognize our status in the medical profession and cooperate to the full with the doctors; this will help to establish a more pleasant relationship between them and the laboratory. We are the "little sisters" of the medical profession rightfully coming into our own, but we must prove ourselves worthy of the trust.

Before discussing methods, let us say a few words about supplies. It pays to buy supplies from well known supply houses. Inferior equipment reduces efficiency fifty percent or more. Pipettes, burettes, etc., which are not calibrated accurately give

erroneous results, and cheap glassware breaks too readily. Trays for blood counts, blood cultures, and the like, should have some type of rack or compartments for the materials needed on them. This makes for neatness and serves to keep the specimens separate for identification; it lessens the chance of getting the specimens mixed. Lack of equipment also lowers efficiency greatly because of the time wasted in hunting around for parts or improvising apparatus. It is well to exercise one's ingenuity at times, but the necessary equipment is essential to efficiency in the laboratory. Borrowing from another department should be limited to special apparatus.

All reagent bottles should be properly labeled, including the name of the reagent, date made and date of expiration if necessary, whether stock or ready for use, the formula, along with the title of the book and page on which it can be found. This refers not only to bottles, but to tubes and other containers as well, and is a point which is commonly neglected.

With regard to methods, the simplest are generally the quickest and, for all practical purposes, as accurate as the more elaborate methods if carefully performed and if the results are interpreted correctly. For instance, the Heller ring test for albumin in the urine, and the micro method for prothrombin time. Simpler methods save time, thus permitting more work to be handled by the laboratory, or a little more time for study or research. Simpler tests also require fewer dishes, which means less work. This is a point to be considered where the "help" problem is one of great importance. This is another point which affects the total efficiency of the laboratory. If the dishwasher has too much, she will not be able to do her work thoroughly, and unclean glassware viciates results.

In this paper we attempted to set forth a few suggestions for increasing efficiency in the laboratory. We sincerely hope that they will prove to be of some help to these who are burdened with the full responsibilities of the laboratory.

REFERENCE

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Footnote for Page 290.

* To Determine the Dilution Factor of Plasma:

FORMULA:	EXAMPLE:
Meniscus Level	5.8 cc.
— RBC Level	— 2.2 cc.
Oxalated Plasma	3.6 cc.
— CC.'s oxalate used	— 1.0 cc.
Total Actual Plasma	2.6 cc.
Dilution factor =	Dilution factor =
Total plasma amount ÷ Total oxalated plasma	2.6 ÷ 3.6 = 0.70

SCIENTIFIC PROJECTS OF THE COLORADO STATE SOCIETY OF MEDICAL TECHNOLOGISTS

The Colorado State Society of Medical Technologists sponsored a new undertaking last year which proved to be both interesting and educational. Each member of the society was invited and urged to participate. The results of our endeavor were exhibited at the National Convention in St. Paul. We want to bring our plans before you at this convention for we feel that some of you might be interested in this type of group activity.

The scientific committee mapped out the program and brought it before the local Denver society at its first monthly meeting of the year. Questionnaires which listed the six projects chosen by the committee were handed out to each member at the meeting and were also mailed to each member in the state. The idea was explained and each one was asked to check a project that would be of interest and all were urged to enter into the group project. There was a provision made for listing other subjects not mentioned in which any might be interested in working out. Each project listed the names of those who would sponsor that particular research problem and would have charge of tabulating the reports and sending out procedure sheets. Several pathologists volunteered to advise and counsel and their names were listed along with the medical technologists who sponsored each project.

Most laboratory workers are too busy with routine procedures to spend much time in individual research. In group participation, however, each one has only to do a few tests and still reap the benefit of the knowledge gained by hundreds of similar tests done in many laboratories throughout the state. This co-operative study made for friendly and interesting communication with outlying members who otherwise would have little professional contact with the larger organized groups.

As soon as the sheets were sent in, procedures were mailed which outlined methods for doing the test chosen along with work sheets for reporting results. All were asked to mail reports in to the sponsor of the project at the end of each month.

This idea being so new, our committee wondered just what response it would receive, if any. The results were very gratifying as you will see by the following summaries. Colorado technologists *are* interested in taking part in constructive co-operative endeavors. Twenty-one laboratories throughout the state participated in this study and reports were sent in by thirty individuals.

The morning session of our state convention was given over

to reports and discussions of the six projects. Some thought that this would be too much time allowed for such reports but there was such an interest shown among the group and so much discussion that the last speaker had to cut her report to a few minutes and the lively morning session was over. If you are looking for a stimulating and profitable program for your group or state meeting, just try out this method of group activity and your problem will be solved.

We shall briefly sketch the final reports which were given on the six projects.

I. Estimation of the Red Cell Count on the Photoelectric Colorimeter.

This is a new procedure to many but is becoming well established in many laboratories across the country. If the colorimeter in use in your laboratory is accurate for determining other colorimetric tests, it can be used satisfactorily for the red cell determination. Three laboratories reported on the test and there are more who are working on it this year. On a series of one hundred and two counts, exclusive of extreme anemias, there were eighty counts which were done on the colorimeter which paralleled those counted on the haemocytometer. The remaining twenty-two showed only slight variation from the 200,000 limit of error allowed in counting red cells. In the group of eighty counts sent in by one large clinic, only two varied slightly from the 200,000 figure. In untreated macrocytic anemias, the count on the colorimeter is higher than the count on the haemocytometer and the reverse is true of the microcytic anemias. This is true because of the variation in the size of the cells. Some of the advantages of the colorimetric method brought out in the final summary were as follows:

1. It takes less equipment.
2. There is less chance for error as much of the human factor in red cell counting is eliminated.
3. It is a great time saver.

II. Comparison of the Basal Metabolism Rate as Calculated by Means of a Factor and Results Obtained by Standard Methods.

This project was considered an interesting one as the formula method of obtaining metabolism results has been discarded by most authorities many years ago. The Gale formula was introduced in the year 1931 by Annabella and G. H. Gale. They based their figures on 1006 cases. They standardized their formula on 20 year old subjects and made corrections according to age. The

factor is based on pulse pressure and pulse rate readings with age corrections. There is a new machine called the Heartometer which is used in some physicians' offices which takes the blood pressure, recording it in chart form. The manufacturers claim that the basal metabolism can be accurately figured from these charts by means of the Gale formula.

The chart compiled by Gale comparing the two methods shows 73.7 percent agreement within a twenty point range in 1000 cases. Technologists do not usually take blood pressures so the pressures which were used to calculate the results in this experiment were not taken by the same individual each time. Therefore, the reports are not uniform. One hundred fifteen reports were sent in. Four cases which used the Heartometer reading for calculating the formula checked exactly with the standard metabolism test. Fifty-nine reports which were figured from the baumanometer readings were within ten points of the metabolor findings. Summarizing the results from the limited number of cases we were able to compare, 76.5 percent were within a twenty point range as compared to the larger reported group of Gale with 73.7 percent within the same range. In the cases that showed only a ten point difference, our results were 5.7 percent higher than the larger group. Only nine of the fifty-nine cases that checked within ten points were out of the normal range of a plus 15 or minus 15. The report concludes that the Gale formula method might well be used as a screening method for calculating the basal metabolism rate where there is no metabolor available.

III. The Sedimentation Rate of Erythrocytes at Different Temperature Readings

Many investigators have written concerning the effect of temperature upon the sedimentation rate and have come to the conclusion that there should be a regulation of the temperature to make for better standardization of the test. If standard temperatures were adopted for setting up this test it would go a long way to bringing out more uniform results.

In this study three temperature setups were employed on each sample of blood. One, at room temperature, one in the incubator and the third was placed in the ice box. Each laboratory used its routine method for performing the test. There were nine hundred and sixty-three tests done on three hundred and thirty-two cases with five different laboratories participating. It was found that temperature does have an effect on the sedimentation rate of erythrocytes. It is evident that it would be beneficial to have a standardized sedimentation rate determination. Temperature is one of the factors as are the type of tube used and the anticoagulant. The important consideration is not that any certain set of

standards be used but that there is more standardization. In this study it was found that normal bloods change in sedimentation rate 0.3 mm per hour for each degree centigraded, while with pathological bloods, the change is 0.6 mm.

IV. Our Fourth Project was a Study of Streptomycin Sensitivity and Methods for the Rapid Growth of the Tubercle Bacillus.

When this subject was brought to the attention of Col. Hugh Mahon at Fitzsimons General Hospital in Denver, he very kindly offered to give a short course to those who were interested. Sixteen of our group joined the class which was held for two hours every week for nine weeks. All who took the course were high in their praise of the instruction they received and there was a good demonstration brought by them to the state meeting. They studied methods of digestion and concentration of sputa, seven different types of culture media for tubercle bacilli, and the resistance of the different strains to Streptomycin.

V. Comparison of the Different Liver Function Tests.

Procedure sheets were sent out for performing the following tests: bromsulphalein, colloidal red, thymol turbidity, and the cephalin cholesterol flocculation. Series of tests were done on cases of infectious hepatitis and also cirrhosis of the liver. There were seven technologists from six laboratories who sent in a total of four hundred and fifty-eight tests. With all the liver function tests there has been found no one test that is specific for liver damage. Each test is an individual one, and only by repeating them at intervals can the progress of the liver damage be determined.

VI. Our Sixth and Last Project Was a Study of the Extent of Glycolysis in Human Blood.

This study was undertaken for the purpose of determining how much sugar is lost from drawn blood if it stands for four hours at room or incubator temperature. We have all been taught that blood which is allowed to stand without laking will soon lose some of its sugar content. Authorities also state that, 1. The enzyme that is responsible for this loss of sugar from the blood is present in the cells and not in the blood plasma, 2. The enzyme is present in a greater extent in the leucocytes than in the erythrocytes and, 4. The activity of the enzyme is dependent upon the pH, temperature, and anticoagulant, and lastly, the enzyme activity is stopped when the blood is laked.

In this study enough blood was withdrawn to make five separate filtrates at intervals of one hour. The first filtrate was made

within five minutes from time of withdrawal. The blood was left without laking between each of the hourly intervals. Each laboratory used the anticoagulant which they routinely use so a comparison of the results of the different methods could be studied. Blood was drawn from both normal and pathological cases. Ten different laboratories and twenty-one technologists reported which make up the following summary:

Average Decrease in Blood Sugar in Four Hours.

1. At room temperature with oxalate (5 bloods) 11 mg. % (range 3-19).
2. Incubator temp. with oxalate (4 bloods) 23 mg. % (range 13-32).
3. Room temp. with heparin (5 bloods) 30 mg. % (range 16-39).
4. Incubator temp. with heparin (2 bloods) 50 mg. % (range 49-52).
5. Diabetics (14 bloods) room temp. 16 mg. %.
6. Leukemia (6 bloods) room temp. 52 mg. % (range 13-123).

The conclusions from this study were that to insure accuracy, the blood sugar determination should be started or the blood laked promptly after the blood is drawn and that incubation of the blood, even in the doctor's vest pocket is not the best procedure.

We are continuing this program in Colorado again this year. Group co-operation brings mutual understanding and educational development and joy of achievement. At the state meeting we are offering a prize for individual projects in addition to group studies. This has meant a lot of work but the results have compensated for the time spent in doing the work and tabulating the results.

Colorado knows it can be done and we hope that we have inspired you to try something similar in your state.

—The Scientific Exhibits Committee, State of Colorado.

AMONG THE NEW BOOKS

DIAGNOSTIC TESTS FOR INFANTS AND CHILDREN. Principles, Clinical and Laboratory Procedures, Interpretation. By H. Behrendt, M.D., 78 tables, 529 pages. New York and London. Interscience Publishers, 1949.

Although this text was prepared primarily for the pediatrician, the wealth of laboratory procedures applicable to pediatric practice places this book in the category of a procedure book. The author has presented his material in such a way that the reader is at all times aware that the subject under consideration is physiology in its application to pediatric practice. Normal and abnormal physiology are discussed; and laboratory tests for detecting abnormality are given which, in the opinion of the author, are most suitable for use. Thus much micro and semi-micro chemistry is given in enough detail to enable a technician to use the book without further reference.

The book is very readable, and remarkable in its clarity.

References have been selected and well chosen.

Those technologists doing considerable work in pediatrics and chemistry will find this a very useful and handy volume to add to the laboratory shelf. The reviewer wishes to predict that this timely volume will find great favor among the modern up to date pediatricians and will soon become a guide for diagnostic procedures.

JORDAN-BURROWS TEXTBOOK OF BACTERIOLOGY. By William Burrows, Ph.D., Professor of Bacteriology, Department of Bacteriology and Parasitology, The University of Chicago with the Collaboration of Francis Byron Gordon, Ph.D., M.D., Biological Department, Chemical Corps, Camp Detrick, Maryland; formerly Professor of Bacteriology and Parasitology, the University of Chicago; and Richard Janvier Porter, Ph.D. Associate Professor of Parasitology, School of Public Health, the University of Michigan and James William Moulder, Ph.D., Assistant Professor of Biochemistry, Department of Bacteriology and Parasitology, the University of Chicago. Fifteenth edition. 264 figures, 981 pages. Philadelphia and London. The W. B. Saunders Company, 1949.

Jordan's Textbook of Bacteriology is as well known as any in the English language; it needs no introduction. This new fifteenth edition has been completely re-written and revised, and thoroughly brought up to date by the splendid group of authors who undertook the revision.

Many strides have been made in bacteriology as a science during the past ten to fifteen years. The material which has accumulated on the subjects of bacterial metabolism and nutrition is voluminous and, unless a technologist were engaged in only bacteriology would it be possible to cover this material.

How fortunate it is to have a text such as this one at hand to provide the fundamental concepts which are accepted as essential to modern bacteriology.

The chapters devoted to viruses and fungi are well written and contain most of the necessary information for general courses in bacteriology and micro-biology. References are well chosen.

Any technician who does not have a recent text on bacteriology would find this volume a definite asset. As a textbook for schools of medicine, nursing, and medical technology there is no book which would fulfill its place any better, as this book is among those texts which have been recognized as firsts on the subject for the past quarter of a century.

BENSLEYS PRACTICAL ANATOMY OF THE RABBIT. By E. Horne Craigie, Ph.D., Professor of Comparative Anatomy and Neurology, University of Toronto, 8th edition, 124 illustrations, 16 plates, 391 pages. Philadelphia and Toronto, The Blakiston Company, 1949. Price $4.25.

This book is not impressive due to its lack of illustrations for a work of this type. At times the style is ambiguous and hard to follow. As a text for comparative anatomy, it would probably meet the demands of the average university course in general mammalian anatomy provided there were adequate laboratory supervision. The fact that the female and male genitalia are not pictured makes this book one which can not be recommended to schools of medical technology as a reference.

SHEARER'S MANUAL OF HUMAN DISSECTION. Edited by Charles E. Tobin, Ph.D., Associate Professor of Anatomy, University of Rochester School of Medicine and Dentistry. 2nd edition, 79 illustrations, 286 pages, 1949. Philadelphia and Toronto, The Blakiston Co. Price $4.50.

This practical manual of human dissection is profusely illustrated; the material tersely presented. Although not intended for the medical stenographer, the reviewer feels that the illustrations are such that by using this text as a reference by those not trained in anatomy, a knowledge of this subject could be gained with considerable ease, thus difficult terms and phrases would be clarified.

Nurses and beginning medical students should find this timely volume a real help in gaining a practical approach to regional anatomy. The editor has succeeded in putting over the point of what is to be expected at the dissection table and also, the illustrations show various anatomical relations that are so necessary in gaining a thorough understanding of anatomy.

All teaching hospitals should provide this volume for their libraries.

BLAKISTON'S NEW GOULD MEDICAL DICTIONARY. Edited by Harold Wellington Jones, M.D., Colonel, U. S. Army, Retired, Contributing Editor, Encyclopedia Americana, Former Director, Army Medical Library, Washington, D.C.; Normand L. Hoerr, M.D., Ph.D., Professor of Anatomy, School of Medicine, Western Reserve University; Arthur Osol, Ph.D., Professor of Chemistry, Director of Chemistry Departments, Philadelphia College of Pharmacy and Science, Editor-in-Chief, United States Dispensatory. With the assistance of an editorial board and over 100 contributors. 1st edition, 252 illustrations, 129 in color; 1294 pages. Philadelphia and Toronto, 1949. The Blakiston Company. Textbook edition $8.50. Thin paper edition $10.75. Deluxe edition $13.50.

In the opinion of the reviewer too much stress can not be placed upon the value of a modern up-to-date medical dictionary. No clinical laboratory should be without one, nor should any up-to-date medical technician feel that a dictionary is unnecessary.

This new Gould dictionary is a monumental work worthy of the highest praise to be given to a work of this kind. Every effort has been made to bring the work up to date, and to provide a simplified system for pronunciation by syllable division and accents. When indicated, phonetic spelling has been used to simplify pronunciation.

The numerous tables and illustrations make this volume the most complete to date, and one which can be highly recommended to all individuals engaged in any branch of the medical profession. No medical school library, nursing school, pharmacy college, hospital, or laboratory is complete without this timely new edition.

TEXTBOOK OF VIROLOGY: For Students and Practitioners of Medicine. By A. J. Rhodes, M.D. and C. E. van Rooyen, M.D. Sc.D. Connaught Medical Research Laboratories and School of Hygiene, University of Toronto, 312 pages, 40 figures, 1949. New York, Toronto, and Edinburgh. Thomas Nelson and Sons.

This text evolved from a series of lectures presented in recent years to the students at the University of Toronto. It is presented as a review of modern advances in the knowledge of viruses and Rickettsae and is intended as a preview for more advanced, postgraduate work. The first chapters are concerned with classification and properties of the viruses, immunity mechanisms and interference phenomena, and the nature, treatment, and spread of virus diseases. From this point the book proceeds with short chapters covering the individual virus diseases from the standpoints of clinical features, pathology, pathogenesis, epidemiology, and some laboratory diagnosis. The chapters on

poliomyelitis, influenza, and encephalitis were particularly interesting.

Students will find this text useful; however, it is the opinion of the reviewer that the text would be of little value for the clinical laboratory worker. There is so little concerning laboratory procedures, and that is indefinite, so that the book would have very little practical use in the average clinical laboratory.

F. M.

ABSTRACT

OBSERVATIONS ON THE STAINING OF CORYNE-BACTERIUM DIPHTHERIAE. By W. Blake Christensen, Laboratory Division, Weld County Health Department, Greeley, Colorado. Stain Technol 24:165 (July) 1949.

After considerable experimentation the author arrived at this formula for diphtheriae staining:

I.	Toluidine Blue, certified (52% dye content)	0.15 gram
	Glacial Acetic Acid	5.00 ml
	Ethyl alcohol, 95%	2.00 ml
	Distilled water	100.0 ml

Filtering is not necessary; the stain may be used as soon as the solution is complete.

II. Albert's Iodine

III. Safranin. (As prepared for gram stain).

Method: Stain smear with Solution No. I for 1 minute, wash with water. Apply Solution No. II for 1 minute, wash with water. Stain with Solution No. III for 15-20 seconds, wash with water. Then dry and examine.

Protoplasmic striations when present are shown, usually staining red with a light pink to practically colorless band of material between. Occasionally these striations are reddish brown The granules stain black and are distinct.

The author feels that this stain uses a single method for demonstrating the finer structure of the diptheriae cell and that the method is practical for college and university students.

FROM THE PRESIDENT

Dear A.S.M.T. Members:

There are so many things that I'd like to talk to you about! For the past two weeks I have been quite busily engaged in visits and meetings for A.S.M.T.

Our Executive Secretary and I visited the Board of Registry office at Muncie for a couple of days. There were many details worked out between that office and ours that will prove most helpful in the future. I wish that each of you could visit their office and see for yourselves what a volume of detailed work is done. You would come away with a keener appreciation of what they are doing for us, I am sure.

The Board of Registry meetings at Chicago followed this visit. We have real news for you from this meeting. A.S.M.T. now has full representation on the Board of Registry—three members. The basis for the choice of these representatives must be decided by the 1950 House of Delegates.

The Seminar fund is to be continued; applications for the use of this fund should be sent to Miss Estelle Downer.

A fund to be used for a national recruitment program was set up in the budget of the Board of Registry. A special committee, headed by Dr. Frank Queen and an A.S.M.T. member, will begin work at once on this program. Miss Ruth Feucht has been appointed to serve with Dr. Queen. You will be hearing from this committee soon. Please cooperate.

A full report of this Board of Registry meeting will be made by Miss Lehman who served as chairman of the Advisory Committee.

As the Journal goes to press, I am visiting the newly established Executive Office in Houston. Since the office has been in operation here for only a month and as I am here for the first day there is little to report to you. All of you will be pleased with its location when you visit it next June.

Sincerely,

Ida L. Reilly, M.T. (ASCP)

COMMITTEE ON NOMINATIONS AND ELECTIONS

To the Affiliated Societies
of Medical Technologists of the
American Society of Medical Technologists & ASMT Officers

Dear Fellow Medical Technologists:

The Nominations and Elections Committee wishes to solicit your suggestions for the offices to be filled at the next annual meeting of the Society.

The offices to be filled at that time and the persons now serving in them are: 1. President-elect—now held by Vernal Johnson; 2. Recording Secretary—now being filled by Sister Eugene Marie; 3. Two members for the Board of Directors to fill the places now held by Oscar Stewart and Jeanne Jorgenson.

The terms of Office for the Board of Directors are for three years. The president elect, after serving in that office for one year, becomes the president of the Society for the following year and a member of the Board of Directors for the year immediately following the presidency. The recording secretary's term is for one year.

May we have your suggestions for these offices, together with the qualifications, previous state or national positions held, and other pertinent information. Remember all candidates must be members in good standing of the American Society of Medical Technologists for at least two years and willing to serve, if elected.

If your Society does not meet before we need this information, we would appreciate having you get in touch with your executive committee and members, where possible, to get their views on this very vital question.

This is your opportunity to tell us whom you want as candidates to fill these offices. Your committee is eager to consider the people whom you suggest. You know who is best qualified in your organization to carry on the affairs of the National Society. You may also have suggestions of persons whom you have met, heard or talked with at our national meetings but who are members of other states. Please mention these, too. We need your recommendations!

We must have your replies before December 1, but we would like to hear from you much sooner. Address replies to Henrietta M. Lyle, Washington County Hospital, Hagerstown, Maryland.

Sincerely,

HENRIETTA M. LYLE, Chairman

Nominations and Elections Committee

NOTICE FROM PROGRAM COMMITTEE

We wish to express our appreciation for the nice response received from the questionnaire sent to all State Presidents regarding Program Material and to ask for your continued support in this. It helps a great deal to know **what kind** of a program you want and **who** can be **depended** on for presentations of same.

PLEASE NOTE THE FOLLOWING AVAILABLE AWARDS FOR AMERICAN SOCIETY OF MEDICAL TECHNOLOGY MEMBERS.

1. Awards from Board of Registry for Scientinc Papers and Scientific Exhibits.

2. ASMT Convention Awards for Scientific Papers and Scientific Exhibits.

3. The Hillkowitz Memorial Contest.

This should be an inspiration to all Medical Technologists to write a Scientific Paper or plan an Exhibit.

Please Note the Following Program Rules:

1. The deadline date for papers to be received by the Program Committee from individuals desiring to present papers in Houston and likewise to compete for the Convention Awards is March 15, 1949.

2. Only ASMT members are eligible to compete for Convention Awards. All competitive papers must be presented in person or by proxy at convention time.

3. All Papers read or submitted to the Society become the property of ASMT and may be published in the American Journal of Medical Technology.

4. The time limit for reading the paper on the Program is 15 to 20 minutes exclusive of showing slides—the remainder of approximately 30 minutes is to be given to discussion.

*Papers may be longer and abstracted for Program purposes.

5. All audio visual aids and professional technicians to operate them will be supplied by the Speakers Supplies Committee, Standard lanterns (3½ by 4) will be furnished unless otherwise requested. Please indicate what aids are needed.

6. **Five** (5) copies of your manuscript must be submitted to the Program Committee Chairman. These must be typewritten, double spaced on regular size typewriter paper.

7. **Two** (2) copies of the manuscript must be submitted by all those NOT COMPETING for ASMT AWARDS and subject to the above instructions.

8. **Prize Papers** from State Contests to be considered for presentation and further awards, must be in the hands of the Program Committee Chairman by March 15, 1950.

Program Committee:

Lucile Harris, Chairman, Hendrick Memorial Hospital, Abilene, Texas;
John N. Frazier, The Street Clinic, Vicksburg, Mississippi;
Martha A. Lee, 5465½ Tilden Avenue, Van Nuys, California;
Homer L. Spencer, Medical Arts Laboratories, Tulsa, Oklahoma, and
Mary Nagai, Box 26, Almeda, Texas.

NEWS FROM THE STATE SOCIETIES

The Association of OREGON Medical Technologists, were host to a Northwest Conference of Medical Technologists from that state, from WASHINGTON, MONTANA, IDAHO (our newest state society), and from British Columbia. Beside the scientific program, there were a number of problems discussed in open forum. The IDAHO group had had a meeting in September. The semi-annual meeting of the MASSACHUSETTS Association was held in Pittsfield, on Saturday, October 22. There were guests from NEW HAMPSHIRE, VERMONT, and NEW YORK. The ALABAMA group met at an "open house" at the Seale Harris Clinic on October 5. They had an attendance of 80. The ILLINOIS Medical Technologists' Association held its annual fall luncheon meeting on October 8. As guests Miss Rachel Lehman, immediate past president of ASMT, Miss Ida Reilly, president, Mrs. Lucille Wallace, ASMT Membership Chairman, and Miss Rose Matthaei, Executive Secretary, ASMT, were present. Their scientific program was followed by a business session.

PRE-CONVENTION THOUGHTS

The SCIENTIFIC EXHIBITS COMMITTEE, headed by Miss Olive Pohlen, 1529 North 5th St., Waco, Texas, is looking forward to arranging for YOUR exhibit to be presented to the crowd that will be attending the ASMT convention in Houston next June. What special work have you done that could be demonstrated in the form of an exhibit for your fellow members of ASMT? Or has your state society pioneered in making a film showing some specific technique, or demonstrating teaching methods? These would be welcome additions to the program.

Miss Lucile Harris has an announcement for the PROGRAM Committee elsewhere in this issue, but some of the leading guest speakers already on the program are Dr. Emma Moss, of New Orleans, Mrs. Thelma Sullivan, of Austin, Texas, Dr. Sunderman, of Houston, Texas (Dr. Sunderman has recently come here from Philadelphia). Papers on Virology, Brucellosis, Amebiasis, Evaluation of Tuberculosis medias, and Medical Technologists in Research, have already been scheduled. The program at this early date is shaping up rapidly to be one of the most interesting and instructive we have ever had. You can't afford to miss it—so start planning now to be in Houston from June 11 through the 15th, 1950.

And the WORKSHOPS (a new wrinkle as far as ASMT is concerned) should be fertile ground for inspiration and ideas. Miss Frieda Claussen and Mr. L. B. Soucy will discuss with one group the various aspects of the work of a Public Relations committee. Miss Ruth Feucht will be in another group, with Mrs. Dorothy Foreman and Mrs. Hortense Leach, to discuss and evaluate recruitment methods. The ASMT Membership Committee, with Mrs. Lucille Wallace as leader, will meet with representatives from the state societies and talk "membership" problems and possibilities. The Executive Secretary, Miss Rose Matthaei, with Miss Mary Eichman, Finance Chairman, hopes that the state treasurers or their representatives will meet in still another Workshop and discuss ways and means of dues collecting, and general finance. Miss Vernal Johnson and Dr. Montgomery will be present to discuss legislation concerning medical technologists in still another group. There will be another workshop where the aspects of socialized medicine touching medical technology will be considered. And Dr. John J. Andujar, of Ft. Worth, Texas, assisted by Miss Rachel Lehman, will discuss training schools and their problems. The above subjects are just a part of the program—you can be thinking about how sorry you'll be if you miss any of it.

Entertainment—oh yes, we'll have enough of that, too. Mrs. Doris

Wallace and her committee have great plans. There will be the usual sight-seeing tours on Sunday afternoon for those who haven't seen Houston before. The early evening will be the hour for the usual reception for getting acquainted, only this will be on the terrace overlooking the lovely Shamrock swimming pool. Monday afternoon there will be a trip to the famous San Jacinto Battle Grounds, where Texas Independence was won in 1836 (how many of you folks outside of Texas knew that this state was once an independent nation, and "joined" the United States, after 12 years as a separate country?) Dinner will be served in the San Jacinto Inn. We hope you like sea food and fried chicken. This is where you'll get it.

On Tuesday evening there will be a barbecue, and perhaps just a bit of a rodeo (pronounced rō'de o, in Texas), for local color. Wednesday, of course, will be all business—for the House of Delegates. And the annual banquet, with all its tradition, is slated for Thursday evening. After Thursday perhaps some of you might like to see more of the Lone Star State, or perhaps go down into Mexico for a post-convention vacation. We'll have the information available.

Then, maybe some of you would like to spend a week or so following the convention on a dude ranch, or even in one of Texas' cities. Beside Houston, there's San Antonio, with its Spanish influence; Dallas, the style center of the South; Ft. Worth, "Cow-town," and a bit of the West; Austin, with the University of Texas, and the hills and beautiful Colorado River. If you would like a vacation on the seashore, there is Galveston. (Shall I tell you a SECRET? Texas has everything—they say that "Texas brags," but just visit us, and see for yourselves that there's a reason). Anyway, we do hope you'll plan to come to the 1950 ASMT Convention at the Shamrock, in Houston, Texas, from June 11 through 15, 1950. (By the way, we mentioned the swimming pool, didn't we? Well, hotel guests have the privilege of using that pool). Shall we plan for all 5000 (Mrs. Wallace tells me that her committee members are working that hard, and are geting so much cooperation from the state membership committee members, that we'll have that number) members to be in attendance? We would like very much to have each of you with us.

HAVE YOU PAID YOUR 1949-50 DUES?

If you have not already paid your 1949-50 ASMT (and State Society) dues, please send your check to your state treasurer TODAY. (See list on page 64 for YOUR state treasurer). According to the Constitution and By-Laws, this NOVEMBER issue of the journal will be your last unless you have paid your 1949-50 dues before the first of the year. (The addressograph plates of all members in arrears with dues are "pulled" at that time, and we don't want to lose YOU as a member).

Please send in address changes promptly. Write both the **old** address and the **new** one on a penny post card (and DON'T forget to put down your name—if you have married recently, please be sure to give us your name as we have it in our files now, as well as your new one). If your journal is not delievered, it is most frequently due to our not having your proper address. Send all address changes to 6544 Fannin St., Houston 5, Texas. (And plan to visit your Executive Office when you are in Houston for the Convention in June.) The Massachusetts Association voted to give the Executive Office $25 for some piece of necessary equipment. We thank you, Massachusetts Medical Technologists.

—The Executive Secretary.

STATE SOCIETIES

ALABAMA: President: Drusilla Mullane, A.P.I., Auburn.
Vice President: Mary Ward, 2904 South 18th St., Birmingham.
Secretary: Mrs. Flora M. Herring, 930 So. 20th St., Birmingham.
Treasurer: Sara Douglas, 212 Mecca Ave., Birmingham.

ARIZONA: No organization.

ARKANSAS: President: Doris Thompson, University Hospital, Little Rock.
President-elect: Betty Rice, P.O. Box 2731, Little Rock.
Vice President: Sara Munn, 1224 Barber, Little Rock.
Secretary: Sister M. James (Poirot), St. Bernard's Hospital, Jonesboro.
Treasurer: Mrs. Naomi Meek, 719 North 34th, Fort Smith.

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Treasurer: Ellen M. Bahr, Birmingham Veterans' Hospital Lab., Van Nuys.

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Secretary: Marguerite B. Pitinga, Memorial Hospital, Colorado Springs.
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Secretary-Treasurer: Sophia A. Rados, State Sanitorium, Sanator.

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